

CLAIMS:

1. A method of prophylactic or therapeutic treatment of fungal infection in an animal or non-aquatic plant comprising administering a sugar acid other than as a metal salt in an amount sufficient to inhibit or prevent fungal growth or reproduction in said animal or non-aquatic plant, wherein said sugar acid in an isolated form or in the form of a biocontrol agent other than *Pseudomonas sp.* strain AN5.

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- 2. The method according to claim 1 wherein the fungal pathogen is selected from the group consisting of: Alternaria spp.; Armillaria mellae; Arthrobotrys oligosporus; Boletus granulatus; Botrytis fabae; Botritis cinerea; Candida albicans; Claviceps purpurea; Cronartium ribicola; Epicoccum purpurescens; Epidermophyton floccosum: Fomes annosus; Fusarium oxysporum; Gaeumannomyces graminis var. tritici; Glomerella cingulata; Gymnosporangium juniperi-virginianae; Microsporum Physoderma alfalfae; Phytopthera infestans; Monilinia fructicola: canis: Pityrosporum orbiculare (Malassezia furfur);Polyporus sulphureus; Puccinia spp.; Т. apiicola; Trichophyton rubrum; Saccharomyces cerevisiae; Septoria mentagrophytes; Ustilago spp.; Venturia inaequalis; and Verticillium dahliae.
- 3. The method according to claim 1 or 2 wherein the fungal pathogen is *G. graminis* (take-all fungus).
- The method according to claim 1 of 2 wherein the fungal pathogen is *Botrytis* fabae.
- 5. The method according to any one of claims 1 to 4 wherein the sugar acid is selected from the group consisting of mannonic acid, gluconic acid, and galacturonic acid.
- 6. The method according to claim 5 wherein the sugar acid is gluconic acid.

- 7. The method according to any one of claims 1 to 6 wherein the sugar acid is administered in pure or partially-purified form.
- 8. The method according to any one of claims 1 to 6 wherein the biocontrol agent is capable of producing an anti-fungal effective amount of the sugar acid when incubated in media containing an aldose substrate.
- 9. The method according to claim 8 wherein the biocontrol agent is a bacterial cell.
- 10. The method of claim 9 wherein the bacterial cell belongs to the genus *Pseudomonas*.
- 11. The method of claim 10 wherein the *Pseudomonas* has the capacity to convert aldose to sugar acid in a PQQ-dependent manner.
- 12. The method of claim 11 wherein the *Pseudomonas* has the sugar acid biosynthesis characteristics of the bacterial strain deposited under AGAL Accession No. NM 00/09624.
- 13. The method of claim 12 wherein the *Pseudomonas* is strain AN5*rif* (AGAL Accession No. NM 00/09624).
- 14. A method of increasing the post-harvest storage of a product from a non-aquatic plant comprising applying to said product a sugar acid other than as a metal salt in an amount sufficient to inhibit or prevent fungal growth or reproduction, wherein said sugar acid in an isolated form or in the form of a biocontrol agent other than *Pseudomonas sp.* strain AN5.

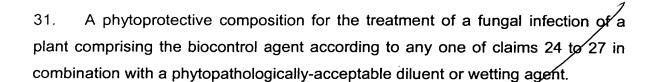
- 15. The method according to claim 14 wherein the sugar acid is selected from the group consisting of mannonic acid, gluconic acid, and galacturonic acid.
- 16. The method according to claim 15 wherein the sugar acid is gluconic acid.
- 17. The method according to any one of claims 14 to 16 wherein the sugar aeid is administered in pure or partially-purified form.
- 18. The method according to any one of claims 14 to 16 wherein the biocontrol agent is capable of producing an anti-fungal effective amount of the sugar acid when incubated in media containing an aldose substrate.
- 19. The method according to claim 18 wherein the biocontrol agent is a bacterial cell.
- 20. The method of claim 19 wherein the bacterial cell belongs to the genus *Pseudomonas*.
- 21. The method of claim 20 wherein the *Pseudomonas* has the capacity to convert aldose to sugar acid in a PQQ-dependent manner.
- 22. The method of claim 21 wherein the *Pseudomonas* has the sugar acid biosynthesis characteristics of the bacterial strain deposited under AGAL Accession No. NM 00/09624.
- 23. The method of claim 22 wherein the *Pseudomonas* is strain AN5*rif* (AGAL Accession No. NM 00/09624).

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- An isolated biocontrol agent for the treatment of a fungal infection in a 24. plant or animal, said agent comprising a bacterial cell other than Pseudomonas strain AN5 having the following characteristics:
- it produces a sugar acid when cultured in the presence of a carbon source (i) comprising an aldose;
- it is capable of colonizing the infection site of said fungus; and (ii)
- it has the biocontrol properties of Pseudomonas strain AN5 rif (AGAL (iii) Accession No. NM 00/09624).
- The biocontrol agent according to claim 24 comprising Pseudomonas strain 25. AN5rif (AGAL Accession No. NM 00/09624) or a derivative thereof.
- The biocontrol agent according to claim 25 wherein the derivative has 26. enhanced capacity compared to Pseudomonas strain AN5rif to produce a sugar acid when cultured in the presence of a carbon source comprising an aldose.
- The biocontrol agent according to claim 25 or 26 wherein the derivative has 27. enhanced capacity compared to Pseudomenas strain AN5rif to colonize the rhizosphere of a plant.
- A phytoprotective composition for the treatment of a fungal infection of a 28. non-aquatic plant comprising an effective amount of a sugar acid in combination with a phytopathologically-acceptable diluent or wetting agent, wherein said sugar acid is in a form other than a metal salt.
- The phytoprotective composition according to claim 28, wherein the sugar 29. acid is selected from the group consisting of mannonic acid, gluconic acid, and galacturonic acid.
- The phytoprotective composition according to claim 29, wherein the sugar 30. acid is gluconic acid.



- 32. The phytoprotective composition according to any one of claims 28 to 31 wherein the wetting agent is a non-ionic detergent.
- 33. The phytoprotective composition according to any one of claims 28 to 32, wherein the fungal infection is an infection by a fungal pathogen selected from the group consisting of Alternaria spp.; A. mellae; A. oligosporus; B. granulatus; B. cinerea; Botrytis fabae; C. purpurea; C. ribicola; E. purpurescens; F. annosus; F. oxysporum; G. graminis var. tritici; G. cingulata; G. juniperi-virginianae; M. fructicola; P. alfalfae; P. infestans; P. sulphureus; Puccinia spp.; S. apiicola; Ustilago spp., V. inaequalis; and V. dahliae; and still more preferably, a fungal pathogen of monocotyledonous plants selected from the group consisting of C. purpurea; G.graminis var tritici; Puccinia spp.; and Ustilago spp..
- 34. The phytoprotective composition according to claim 33 wherein the fungal pathogen is *G. graminis* (take-all fungus).
- 35. The phytoprotective composition according to claim 34 wherein the fungal pathogen is *Botrytis fabae*.
- 36. A composition for the treatment of a fungal infection in a human or other mammal comprising an effective amount of a sugar acid in combination with one or more pharmaceutically-acceptable carriers or diluents, wherein said sugar acid is in a form other than a metal salt.
- 37. The composition according to claim 36 wherein the sugar acid is selected from the group consisting of mannonic acid, gluconic acid, and galacturonic acid.

- 38. The composition according to claim 37, wherein the sugar acid is gluconic acid.
- 39. A composition for the treatment of a fungal infection in a human or other mammal comprising the biocontrol agent according to any one of elaims 24 to 26 in combination with one or more pharmaceutically-acceptable carriers or diluents.
- 40. The composition according to any one of claims 36 to 39 for the treatment of a condition selected from the group consisting of tinea pedis (athlete's foot), tinea cruris, tinea corporis (ringworm), candidiasis, onychia, paronychia, external genital candidiasis, candidal balanitis, pityriasis versicolor and jockey-strap itch.
- 41. A method of producing a sugar acid comprising incubating a bacterial cell having the biocontrol properties of *Pseudomonas* strain AN5 *rif* (AGAL Accession No. NM 00/09624) in the presence of aldose for a time and under conditions sufficient for PQQ-dependent oxidation of the aldose to sugar acid to occur, wherein said bacterial cell is not *Pseudomonas* strain AN5.
- 42. The method according to claim 41 wherein the bacterial strain is Pseudomonas strain AN5 rif (AGAL Accession No. NM 00/09624).
- 43. The method according to claim 41 or 42 wherein the aldose is selected from the group consisting of glucose, mannese and galactose.
- 44. The method according to claim 43 wherein the aldose is glucose.
- 45. The method according to any one of claims 41 to 44 wherein the culture conditions comprise growth on potato dextrose media or pontiac medium containing aldose substrate.

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- 46. The method according to claim 45 wherein the aldose substrate is at about 2% (w/v) to about 4% (w/v) in the culture medium.
- 47. The method according to any one of claims 41 to 44 wherein the culture conditions comprise growth on potato dextrose media or pontiac medium followed by incubation in a concentrated solution of aldose substrate.
- 48. An isolated nucleic acid molecule which comprises a nucleotide sequence which encodes one or more enzymes involved in the biosynthesis of a sugar acid, wherein said nucleotide sequence is selected from the group consisting of:
 - (i) a nucleotide sequence which is at least about 50% identical to at least about 30 contiguous nucleotides of SEQ ID NO: 1 or a complementary sequence thereto;
 - (ii) a nucleotide sequence which is at least about 50% identical to at least about 30 contiguous nucleotides of the *Pseudomonas* gene sequence contained in the cosmid clone pMN M53 (AGAL Accession No. NM 00/09622);
 - (iii) a nucleotide sequence which is capable of hybridising under at least low stringency conditions to at least about 30 contiguous nucleotides of SEQ ID NO: 1 or a complementary sequence thereto;
 - (vi) a nucleotide sequence which is capable of hybridising under at least low stringency conditions to at least about 30 contiguous nucleotides of the *Pseudomonas* gene sequence contained in the cosmid clone pMN M53 (AGAL Accession No. NM 00/09622); and
 - (vii) a nucleotide sequence which is degenerate to SEQ ID NO: 1 or the *Pseudomonas* gene sequence contained in the cosmid clone pMN M53 (AGAL Accession No. NM 00/09622).

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- 49. The isolated nucleic acid molecule of claim 48 wherein the nucleotide sequence encodes a sugar oxidase enzyme.
- 50. The isolated nucleic acid molecule of claim 49 wherein the sugar oxidase enzyme is a PQQ-dependent sugar oxidase enzyme.
- 51. The isolated nucleic acid molecule according to any one of claims 48 to 50 comprising the nucleotide sequence of SEQ ID NO: 1.
- 52. The isolated nucleic acid molecule according to any one of claims 48 to 51 comprising the nucleotide sequence of the *Pseudomonas* genome contained in the cosmid clone pMN M53 (AGAL Accession No. NM 00/09622).
- 53. An isolated nucleic acid molecule comprising a nucleotide sequence encoding one or more enzymes involved in the biosynthesis of PQQ, wherein said nucleotide sequence is selected from the group consisting of:
- (i) a nucleotide sequence having at least about 50 contiguous nucleotides of any one of SEQ ID NOs: 2 to 6 or a complementary sequence thereto;
- (ii) a nucleotide sequence having at least about 50 contiguous nucleotides of the *Pseudomonas* gene sequence contained in the cosmid clone pMN-L2 (AGAL Accession No. NM 00/09621); and
- (iii) a nucleotide sequence that is degenerate to any one of SEQ ID NOs: 2 to 6 or the *Pseudomonas* gene sequence contained in the cosmid clone pMN-L2 (AGAL Accession No. NM 00/09621).
- 54. The isolated nucleic acid molecule according to claim 53 comprising the nucleotide sequence of SEQ ID NO: 2.
- 55. The isolated nucleic acid molecule according to claim 53 comprising the nucleotide sequence of SEQ ID NO: 3.

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- The isolated nucleic acid molecule according to claim 53 comprising the 56. nucleotide sequence of SEQ ID NO: 4.
- The isolated nucleic acid molecule according to claim 53 comprising the 57. nucleotide sequence of SEQ ID NO: 5.
- The isolated nucleic acid molecule according to claim 53 comprising the 58. nucleotide sequence of SEQ ID NO: 6.
- The isolated nucleic acid molecule according to any one of claims 53 to 58 59. comprising the nucleotide sequence of the Pseudomonas genome contained in the cosmid clone pMN-L2 (AGAL Accession No. NM 00/09621).
- A method of producing a sugar acid comprising expressing the isolated 60. nucleic acid molecule according to any one of claims 48 to 52 in a cell, tissue or organism and culturing said cell, tissue or organism in the presence of an aldose substrate for a time and under conditions sufficient to produce a sugar acid.
- The method according to claim 60 further comprising introducing the nucleic 61. acid molecule to the cell, tissue or organ in a expressible format.
- The method according to claim 60 or 61 further comprising extracting or 62. purifying the sugar acid produced.
- The method according to any one of claims 60 to 62 wherein the cell is a bacterial cell.
- The method according to claim 63 wherein the bacterial cell is a 64. Pseudomonas sp.

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- 65. The method according to any one of claims 60 to 62 wherein the cell, tissue or organ is a plant cell, tissue, or organ.
- 66. The method according to any one of claims 60 to 65 further comprising expressing the nucleic acid molecule according to any one of claims 53 to 59 for a time and under conditions sufficient to produce PQQ in the cell, tissue or organ.
- 67. A method of enhancing the tolerance of a plant to infection by a fungal pathogen comprising expressing therein the isolated nucleic acid molecule according to any one of claims 48 to 52, and optionally a second isolated nucleic acid molecule encoding one or more PQQ-biosynthesis enzymes, for a time and under conditions sufficient for a sugar acid to be produced by said plant, or by a cell, tissue or organ of said plant.
- 68. The method according to claim 67 wherein the second isolated nucleic acid molecule is the nucleic acid molecule according to any one of claims 53 to 59.
- 69. A transformed plant comprising the isolated nucleic acid molecule according to any one of claims 48 to 59.
- 70. A progeny plant, cell, tissue or organ of the plant according to claim 69, wherein said progeny, cell, tissue or organ comprises the isolated nucleic acid molecule according to any one of claims 48 to 59.